



Figure 6.4. Localization of biochemical mechanisms of cellular respiration: (1) glycolysis in the cytosol; (2) citric acid cycle in the mitochondrial matrix; (3) electron transport and oxidative phosphorylation in the cristae.

of a basic respiratory assembly. Their objective was to determine the structure of one of these units, which they pursued through a series of experiments that focused selectively on the reaction between cytochrome *c* and oxygen (Cooper & Lehninger, 1956a; Cooper & Lehninger, 1956b; Devlin & Lehninger, 1956), on ATPase activity (Cooper & Lehninger, 1957a), and on ATP- P_i ³² and ATP-ADP exchange reactions (Cooper & Lehninger, 1957b). The exchange reactions involved the regular exchange either of a free phosphate with one in ATP or the transfer of a phosphate from an ATP molecule to an ADP molecule. All of these reactions occurred in the digitonin prepared particles and were inhibited by decoupling agents. From these studies they concluded that phosphate and ADP enter into oxidative phosphorylation in separate, sequential steps. By investigating the exchange reactions in particular, Lehninger and his colleagues claimed to determine the order of the final events in phosphorylation. The alternatives were that the responsible enzyme (they used X, Y, and Z to represent the enzymes involved in each of the phosphorylation reactions) first reacted with the phosphate, creating a high-energy intermediate $X\sim P$, etc., and then reacted with ADP to form ATP, or that they reacted first with ADP, creating $X\sim ADP$, etc., and then with the phosphate. The evidence from the exchange reaction supported the former, not the latter, possibility (see Figure 6.5).